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Toxicology, biosynthesis, bio-control of aflatoxin and new methods of detection

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ABSTRACT

Mycotoxins and their derivatives since their discoveries and until the present time are behind unspecified economic and medical damages. Aflatoxins are classified according to their physical–chemical and toxicological characters in the most dangerous row of the mycotoxins. These aflatoxins are in part responsible, of irreversible medical disasters that are not easily manageable such as cancer of the liver and kidneys, and in the other part, of losses in the stored cereal products. Based on these crucial findings, monitoring of this toxin became imperative in post-harvest food products, during storage, during trans-formation chain and even during the long phases of conservation. Vigilance of this toxin is delivered by detection methods using very advanced technologies to respond in the shortest possible times. In addition, the knowledge of factors supporting the biosynthesis of aflatoxins such as the temperature, moisture content, concentration of nitrogen and carbon, and the molecules responsible for the genetic control of the synthesis will be reflected later in the choice of bio-control techniques. This control is currently based on new strategies using the bioactives substances of the plants, the lactic bacteria and some strains of actinomycetes that have good inhibiting activity against aflatoxins with fewer side effects on Man. On the other hand, this brief review summarizes the results of new studies demonstrating the toxicity of the toxin, new detection methods and bio-control.

1. Introduction

Mycotoxins are a much diversified group of toxic compounds produced by five kinds of spore-forming fungi, known to cause noxious effects to the health of human and animals. Food security is regularly risked by mycotoxins appearing in food [1,2].

Amongst the mycotoxins, aflatoxins are most intensively sought because of their immunotoxicity acting on phagocytes and cell-mediated immunity [3]. They are regarded as natural contaminants from a large variety of agricultural products such as maize [4]. These compounds also affect a wide range of foods and fermented foods because of their richness in nutrients promoting their syntheses [5,6]. Their threshold can exceed the standards set by the European Union as in dry sweet chestnut occasionally consumed [7,8].

The producing fungi of these types of mycotoxins can develop inside some food when the environmental conditions are favorable to the biosynthesis [9]. Slightly higher CO_2 concentrations, interactions with the temperature and the availability of water can stimulate the growth of some mycotoxigenic species, especially under hydrous stress [10].

Aflatoxins are mainly produced by the moulds belonging to the species *Aspergillus* [11–14] such as *Aspergillus flavus* (*A. flavus*), *Aspergillus nomius*, and *Aspergillus parasiticus* (*A. parasiticus*) [15]. Aflatoxins B_1, B_2, G_1, and G_2 are most significant contaminants to rice [16]. Two other metabolites: aflatoxins M_1 and M_2 can be separated from milk [17,18].

These poisons cause very dangerous effects in the consumer: these include carcinogenic, mutagen and teratogenic effects [19]. Consumption of aflatoxins contaminated corn has been found associated with an increased risk of cancer of the liver and acute hepatitis in certain areas of the South Africa and China [20,21]. A study showed that the consumption of groundnuts contaminated by aflatoxins caused the death of several cows with hepatic damage according to the histological analysis [22]. Strong exposure to aflatoxins causes growth delay in young children [23].

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Aflatoxin B_1 (AFB_1) is the most relevant mycotoxin because of its toxic effect in humans [13], it is found with higher concentrations in contaminated food [24]. AFB_1 is particularly toxic because of its role in liver cancer [17,18].

The objective of this literature study is to collect maximum information and clarifications argued with recent references to present a bibliographical review covering the toxicological profile of AFB_1, the new methods of bio-control and recent techniques available for detecting this toxin.

2. Recent studies showing the toxicity of AFB_1

AFB_1 is a powerful carcinogen harmful to health as it causes lung and liver cancer [25,26]. The International Agency for Research on Cancer recognized that AFB_1 and aflatoxin M_1 are carcinogenic from Group 1 for human and animals [27].

Recent studies carried out on the mice showed that the early exposure to the AFB_1 in particular at the embryonic period is a mutagen [28], as it causes a reduction of the body weight, a reduction in the weight of the reproductive organs, a reduction in the number and mobility of the spermatozoa with a lowering of the rate of serum testosterone and the enzymes of the steroidogenesis [29].

The cytotoxicity was shown *in vivo* on the renal cells of a monkey. The results showed that this toxin causes a considerable reduction in the viable cells (37%), it also causes oxidative damage by enhancing the peroxidation of lipids [30]. In Brazil, aflatoxin M_1 was detected in the human urines with a going rate from 0.19 to 12.7 pg/mg, whereas no residues of aflatoxins B_1, B_2, G_1 and G_2 were identified [31]. The AFB_1 is toxic for human lymphocytes mediated by apoptosis and necrosis [32].

In broiler chicken aflatoxins affect the pancreatic activity, resulting in histological changes of the organ, with an increase in the activity of lipase and alpha amylase, and the activity of trypsin is also affected [33].

3. Major factors influencing biosynthesis of AFB_1

The biosynthesis of the AFB_1 requires several steps (Figure 1) and it is perhaps affected by the intervention of several environmental factors (stress, quorum sensing and protein signaling pathway) without forgetting the factors regulating the transcription unit [34].

Amino-acids such as tryptophan inhibit the synthesis of aflatoxin whereas tyrosin encourages it [35]. The presence of the lipids induces the aflatoxinogenesis [36]. Among the organic factors affecting biosynthesis, carbon and nitrogen are the major ones [37]. In addition, simple sugars such as glucose and fructose support this biosynthesis, whereas in the cases of sorbose and lactose no action has been recorded [38].

Concerning the physical factors, the optimal temperature of biosynthesis is located between 28 °C and 35 °C. Above this temperature range, biosynthesis is inhibited due to the attack of transcription genes *aflR* and *aflS* [39,40], whereas under the conditions of dryness, the production of the aflatoxins is high [41]. Synthesis is also influenced by subcultures and changes in the morphology of producing cells [42]. For pH, biosynthesis is high in acidic mediums while it is inhibited in basic conditions [43], for *A. parasiticus*, the growth in water is faster with a pH ranging from 5.5 to 6.5 [44].

The secondary plant metabolites play a key role in the synthesis of aflatoxins [45]. For example, the presence of the octanal causes a reduction of 60% of the fungic growth with a rate of increase in the production of aflatoxins of 500% [46]. However, hydrolysable tannins considerably inhibit the biosynthesis of aflatoxins [47]. Some antioxidants such as the phenolic compounds, ascorbic acid and caffeic acid decrease, in an important way, the aflatoxinogenesis, without any effect on the growth of the fungi [48,49].

4. Detoxification and bio-control of aflatoxins

Harmful effects caused by this dangerous toxin have directed researchers towards finding new strategies for prevention and detoxification in order to preserve the safety of products intended for human consumption [50].

4.1. Detoxification using probiotics and lactic acid bacteria

Several lactic bacteria are able to bind AFB_1 *in vitro* and *in vivo* on the surface of the organism and take two aspects were into consideration: binding and release of toxin [51]. Turbic and his collaborators showed that 77%–95% of AFB_1 were removed by strains of *Lactobacillus rhamnosus* GG and LC-705 [52]. El Khoury and his collaborators also noted that *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were effective in the reduction of aflatoxins M_1 [53]. *Lactobacillus pentosus* and *Lactobacillus beveris* have the capacity to absorb and release AFB_1 [54].

The activity of *Lactobacillus plantarum* (*L. plantarum*) is studied in Tunisia on olives (Chetoui varieties) collected in 2008–2009 and 2009–2010. Samples were prepared and then inoculated by *A. flavus* and *L. plantarum*. Results of crude samples showed that samples of 2008–2009 were salubrious due to the presence of antimicrobial substances and the absence of the biosynthesis of this toxin [55]. The identification of mycoflore revealed presence of species belonging to genus *Candida*, *Rhodotorula*, *Crytococcus*, *Pichia*, *Aspergillus*, *Geotrichum*, *Penicillium* with absence of AFB_1 producing species. For those of 2009–2010 and at a rate of 2.10^6 cell/g of *L. plantarum* AFB_1 was reduced from 11.0 µg/kg up to 5.9 µg/kg [55].

L. plantarum adheres to the surface of olive and produces biofilms, which affects adherence of other undesirable microorganisms, supports the increase in antioxidant activity and consequently, it weakens the production of AFB_1 [55]. This reduction is also due to attraction of oxygen by *L. plantarum* thus protecting of polyols against oxidation and increasing inhibition of AFB_1 biosynthesis [55], According to other studies, such reduction of AFB_1 rate is due to the binding of toxin by reversible physical bonds [56], binding with certain molecules in the wall of the *L. rhamnosus* GG [57], or by synthesis of extracellular polysaccharides trapper of radicals and having an antioxidant activity in some probiotics like *Bacillus coagulans* RK-02 [58]. Other strains of *Bacillus* spp. have the ability to degrade AFB_1 [59].

According to Magnusson and his collaborators, three mechanisms can explain the antimicrobial effectiveness of the lactic bacteria, namely, organic acid production, competition for nutritive element and antagonist production [60].
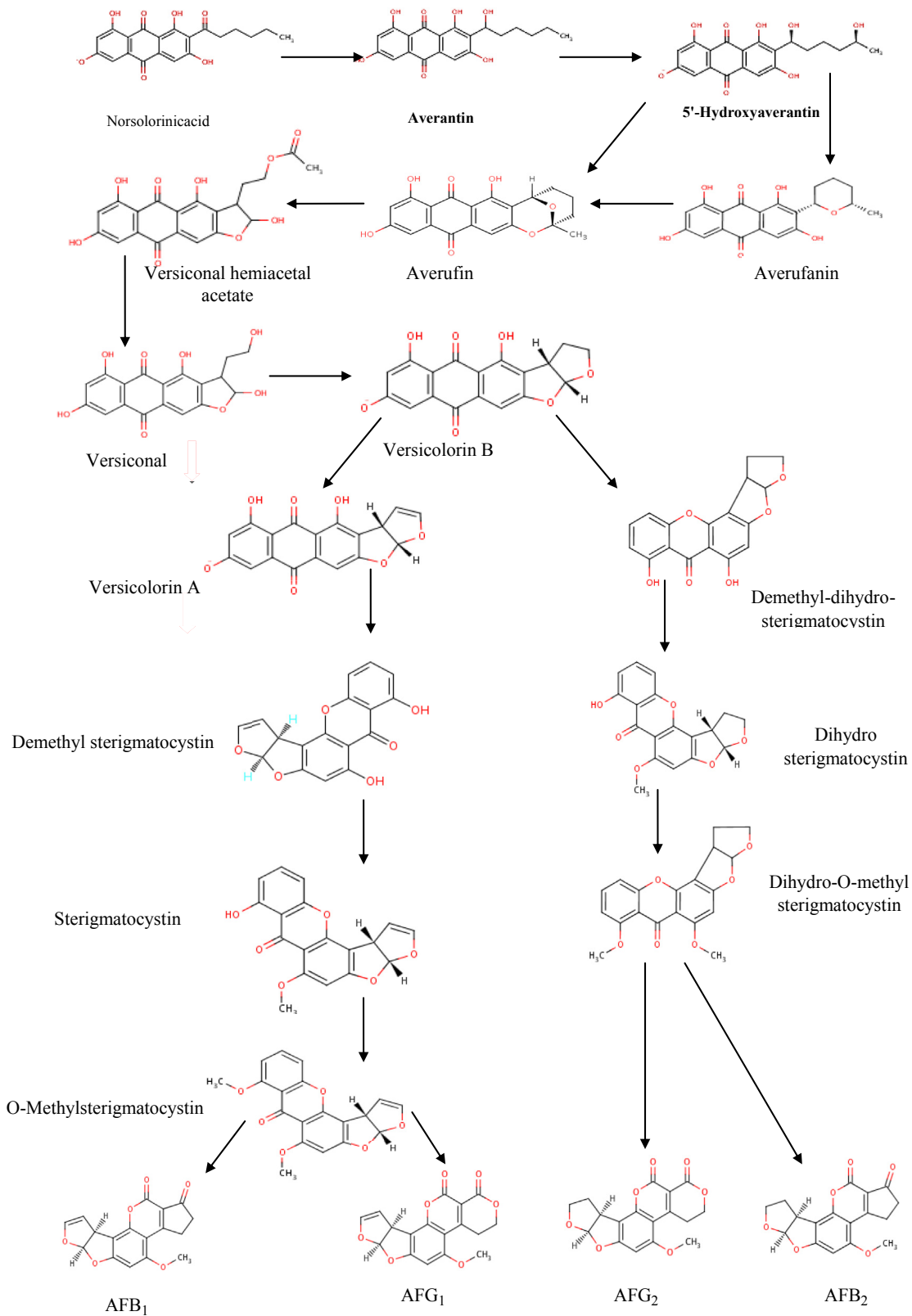
Figure 1. Biosynthesis steps of aflatoxin.

4.2. Detoxification using bioactive substances of plants

Phenolic pulp extract of *Dialium guineense* has been proved effective for sweeping and trapping of oxygenated chemical species and the prevention of lipid peroxidation, proteins oxidation and DNA fragmentation by AFB_1 [61]. Another adsorbent prepared from bagasse has been proved effective for detoxification of AFB_1 in the gastrointestinal tract of chicks and no negative symptoms was reported [62].

Aflatoxin oxidase, an enzyme of *Armillariella tabescens* presents a detoxifying activity towards AFB_1. This reaction is dependant on oxygen and hydrogen peroxide producing, which may play the crucial role in detoxification of aflatoxin oxidase [63]. Laccase is another enzyme that has proven its detoxifying affinity for AFB_1 [64]. Furthermore, manganese peroxidase is an enzyme of *Pleurotus ostreatus* which may detoxify AFB_1 according to the enzyme concentration and the incubation period, this detoxification can reach 90% at 1.5 IU/mL of enzyme during 48 h of incubation [65]. The treatment with kaempferol also decreases toxic effects of AFB_1 [66].

4.3. Detoxification using biomolecules of fungi

Fungal proteins are molecules of small sizes very basic and rich in cysteine such as PgAFP, NFAP and PC-Arctin [67–69]. The NFAP induces an oxidative stress in sensitive fungi causing the apoptosis [70], whereas, PgAFP inhibits growth in some toxigenic molds [67]. A recent study proved a reduction growth of *A. flavus* with significant changes including several proteins at concentration higher than 9.38 μg/mL of PgAFP. Cells treated by PgAFP showed a more intense oxidation [71].

4.4. Detoxification using actinomycetes

New control strategies have been developed in recent years based on the use of actinomycetes. A study conducted on Cuban soil led to isolation of 563 actinomycetes, in which 50.7% have an antifungal activity against *Candida, Trichophyton menta-grophytes, Penicillium chrysogenum* and *Colletotrichum musae*, probably due to the production of antibiotics belonging to the families of aminoglycoside, anthracycline or polyether iono-phore and polyene macrolide antibiotic [72]. Another study conducted by Okudoh and Wallis in 2007 revealed that actinomycetes isolates from forests soil had an antimicrobial activity better than those from the riparian soil. Isolates from poultry manure, straws, chickens and composts soil had inhibition zones varying between 20 and 30 mm against *Candida utilis* [73].

In 2013, a study showed that *Streptomyces phaeochromo-genes* isolated from soil of a garden of Sagar, Madhya Pradesh showed a very good inhibiting activity against *Candida albicans* due to an intracellular antibiotic with a 27 mm inhibition zone [74]. In addition, a new strain identified and named *Streptomyces* sp. PP14 has just been isolated from the Canadian soil after their thermal and chemical treatment with phenol in order to have resistant strains. This strain showed a very good activity against *Penicillium expansum, Fusarium graminearum, A. flavus* and *Aspergillus carbonarius* with inhibition zones varying between 17 and 45 mm on solid medium ISP2 [75]. Recently, a study allowed to isolate 37 strains of actinomycetes from various areas of Algeria. These strains showed a very good activity against *A. flavus* NRRL 62477. Their culture in a medium containing 5 mg/kg of pure AFB_1 allowed to have a reduced rate which can go up to (15.6 ± 11.7) AFB_1 (residual concentration in the medium in %) [76].

4.5. Detoxification using other methods

A study was performed on 192 laying hens in order to evaluate the effect of vitamin E on AFB_1 administration. The results revealed that eggs production was reduced completely after three weeks of exposure to AFB_1 (10 μg/kg) and vitamin E (0.1 mg/kg), the shell thickness was less than normal, AFB_1 was present in eggs at 2.5 μg/kg despite of the presence of vitamin E, the latter recorded an antioxidant effect against hemolyses caused by 2,2′-azobis (2-amidino-propane) hydrochloride [77].

Another study was carried out on six groups of Swiss albino mice in order to determine the protective effect of esculin and ascorbic acid against AFB_1, and it showed that AFB_1 causes 387% of lipid peroxidation of necrosis and degeneration in renal tubules. The addition of esculin or the ascorbic acid decreased this effect, which is due to on the one hand the controlling of reduced glutathione, glutathione peroxidase, glutathione-S-reductase, glutathione reductase, superoxide dismutase and catalase and on the other hand, the regeneration of the renal tubules and the glomerular epithelial cells [78].

5. New methods of detection of the AFB_1

Detection methods of AFB_1 has underwent remarkable development since its discovery. Thin layer chromatography is one of the oldest techniques used to analyze contaminated samples [79]. Other methods are also used such as high performance liquid chromatography with fluorescent detector [80], or with fluorimetric detector [81]. Aflatoxins are also detected by liquid chromatography coupled to a mass spectrometer [82]. Other methods of detection were elaborated such as immune-affinity column immune-enzymatic and immunochemical methods [83,84].

Detection of ultra-traces of AFB_1 is extremely important for food safety, this detection requires very powerful techniques. Among the preview of these techniques during this bibliographic research, an aptasensor using unmodified gold nanoparticules indicator based on the aggregation phenomenon of gold nano-particules induced by salt was recently developed [85]. Another very recent technique by electrochemical immunosensor sensitive to AFB_1 based on carbon nanotubes with simple walled, this immunosensor was based on an indirect competitive binding. The detection limit is 3.5 pg/mL. In addition, the immunosensor was successfully applied for determination of AFB_1 in corn powder, which showed a good correlation with the results obtained by high performance liquid chromatography [86].

Technique ultra-high pressure liquid chromatography tandem mass spectrometry was developed to identify and quantify simultaneously mycotoxins in ensiling grasses. It was performed using a modified QuEChERS extraction by employing an acidified aqueous extraction [87]. Another uncomplicated technique with a detection limit of 0.03 ng/mL for AFB_1 based on sensitive surface-enhanced Raman scattering beacons has been developed without nucleic acid amplification [88].

Other ultra-sensitive strategies; colorimetric and homogeneous for AFB_1 detection were set up using a DNA aptamer, and two halves of split DNAzyme has been developed [89].

Another inexpensive method, free from any interference and even automated has been developed successfully. This technique is used for the detection of AFM1 in milk based upon micro-extraction in the liquid phase of hollow fiber coupled with the liquid chromatography/tandem mass spectrometry [90]. Visual detection method has undergone a remarkable development. In this review, a visual detection method was implemented using chemical reactions with dichlorvos-ammonia on agar cultures, wherein dichlorvos inhibits esterase causing accumulation of anthraquinone precursors of aflatoxins mycelium to agar, followed by a change in the color of colonies from light yellow to purple red by treatment with ammonia vapor [91].

In order to facilitate the bio-monitoring which provides the best approach to evaluate the human exposure to the mycotoxins, the detection of AFM1 in urines (derived from the decomposition of AFB_1) was performed by the immune-enzymatic method [92].

6. Conclusions

In this literature review, all information collected were obtained from very recent studies. The information presented in this paper has demonstrated the toxic effects of AFB_1. Bio-monitoring and bio-control are two crucial points in order to limit all undesirable effects or to make them reversible.

Conflict of interest statement

We declare that we have no conflict of interest.

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